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(57) Abstract

Infections in children caused by a primary immunodeficiency of cellular immune function in the child may be treated by administering to the child an immunotherapeutically effective amount of interleukin-2 (IL-2). The immunodeficiency is typically severe combined immunodeficiency syndrome or common variable immunodeficiency. The patient must have a cellular immune function that can be shown to be improved *in vitro* through the addition of IL-2 to blood cells taken from the patient.

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TREATMENT OF INFECTIONS CAUSED BY A PRIMARY IMMUNODEFICIENCY WITH INTERLEUKIN-2

The present application is a continuation-in-part of copending U.S. Serial No. 125,380, filed November 25, 1987, which is hereby incorporated by reference in its entirety.

Background of the Invention

Field of the Invention

This invention relates to the field of medical treatment. More particularly, this invention is directed to a method for treating opportunistic infections that result from primary immunodeficiencies of cellular immune function in human pediatric patients.

Description of Related Art

The primary immunodeficiencies of cellular immune function are a heterogeneous group of disorders in children in which one or more mechanisms of immunity in the human infant are deficient. This disease is characterized by severe dysfunction of both the T cell and B cell systems. Affected individuals may have recurrent and chronic opportunistic infections, such as bacterial, fungal, and viral infections.

Attempts to classify the various primary immunodeficiencies have been made. See, for example, WHO (1979) "Immunodeficiency report of a WHO scientific group", Clin. Immunol. Immunopathol. 13:296. Such attempts, however, are difficult at best because of the rapid progress in the technology used to identify cells and subsets of cells that comprise the immune system. Examples of types of primary immunodeficiencies include severe combined immunodeficiency syndrome (SCIDS) and common variable immunodeficiencies (CVID). In addition, analysis of the immunodeficiency demonstrates a profound depression in the number and function of T cells and B cells and a concomitant defect in the synthesis of the immunoenhancing lymphokine, interleukin-2 (IL-2). Some types of immunodeficiencies are IL-2 dependent, in that they show a defect in mitogen-

induced lymphocyte proliferation that is at least partially corrected or restored when IL-2 is added to the lymphocytes in vitro. Other types do not show such correction upon addition of exogenous IL-2.

Many of the primary immunodeficiencies are genetically determined disorders; however, the non-heritable forms of these diseases are clinically indistinguishable.

Before the advent of bone marrow transplant capabilities, the majority of patients with severe immunodeficiencies died before their second birthday. Bone marrow transplant has become the treatment of choice for those patients with HLA-MLC matched donors. Ninety percent or greater of patients treated in this manner will achieve lasting restoration of immunocompetence. Unfortunately, patients who do not have HLA-MLC histocompatible donors achieve a lower transplant success rate and may require frequent intervention to control infections and maintain an adequate nutritional status.

IL-2, a lymphokine that is produced by normal peripheral blood lymphocytes and induces proliferation of antigen- or mitogen-stimulated T-cells after exposure to plant lectins, antigens, or other stimuli, was first described by Morgan, et al., Science,193:1007-1008 (1976). In addition to its growth factor properties reported by Morgan et al., IL-2 has been found also to modulate a variety of functions of immune system cells in vitro and in vivo.

IL-2 was initially made by cultivating human peripheral blood lymphocytes (PBL) or other IL-2-producing cell lines. See, for example, U.S. Patent No. 4,401,756. Recombinant DNA technology has provided an alternative to PBLs and cell lines for producing IL-2. Taniguchi, T. et al., Nature (1983), 302:305-310 and Devos, R., Nucleic Acids Research (1983), 11:4307-4323 have reported cloning the human IL-2 gene and expressing it in microorganisms.

U.S. Patent No. 4,518,584 describes muteins of IL-2 in which the cysteine normally occurring at position 125 of the wild-type or native molecule has been replaced with a neutral amino acid, such as serine. These muteins possess biological activity. U.S. Patent No. 4,604,377 issued August 5, 1986 discloses an IL-2 composition suitable for reconstituting in a pharmaceutically acceptable aqueous vehicle composed of oxidized microbially produced recombinant IL-2. The IL-2 is

noted as useful inter, alia, in the treatment of immunodeficiency states (acquired, inborn, or induced) and of infection.

Various therapeutic applications of human IL-2 have been investigated and reported by S. Rosenberg and colleagues (see, for example, Mule et al., Science (1984), 225:1487, S. Rosenberg et al., New England Journal of Medicine (1985), 313:1485-1492, and U.S. Patent No. 4,690,915 issued September 1, 1987).

In addition, it is known from the literature that IL-2 may be used to treat immunodeficient conditions (see, e.g., Chang et al., J. Biol. Response Mod., 3:561-572 (1984), Merluzzi and Last-Barney, Int. J. Immunopharmacol. 7:31-39 (1985), Kolitz et al., Arzneimittelforschung, 35:1607-1615 (1985); Fahey et al., Ann. Intern. Med., 106:257-274 (1987) (immunodeficiency disorders); WO 86/00334 published 1/16/86 (Takeda); EP 132,359 published 1/30/85 (Takeda); W. German Pat. Appl. No. 3242851 published 5/24/84 (GBF GesBiotech); and EP 92,163 published 10/26/83 (Sloan-Kettering Institute)).

The effect of exogenous IL-2 on the in vitro T cell responses of human patients with primary deficiencies of T cell function has been studied by Flomenberg and his colleagues (see Flomenberg et al., J. Immunol. 130:2635-2643 (1983) and Flomenberg et al., J. Immunol., 130:2644-2650 (1983)). Only one in five patients studied had lymphocytes responsive to exogenously supplied IL-2.

No marked clinical improvement upon use of exogenous IL-2 in vivo was apparent in two separate studies of patients with immunodeficiency states. See, e.g., Dopfer et al., Immunobiology, 167:452-461 (1984) and Welte et al., in Sorg C. et al., ed., Cellular and Molecular Biology of Lymphokines; International Lymphokine Workshop 4th, 1984, Schloss Elmau, West Germany (Orlando: Academic Press, 1985), p. 755-759.

A human growth cell factor distinct from IL-2 and described as useful for treating SCIDS and acquired immunodeficiency syndrome (AIDS) is reported in EP 163,603 published 12/4/85 (Sandoz).

Studies have been proposed or carried out for using IL-2 in the treatment of human immunodeficiency virus (HIV) infection responsible for AIDS. (See, e.g., Siegel et al., Infection, 12:298-302(1984); Siegel et al., Infection, 13:S219-S223 (1985); Mitchell, Hum. Pathol., 16:97-98 (1985); Gramatzki et al., Immunobiol.,

172:438-447 (1986); Gramatzki et al., Klin Wochenschr., 65:380-386 (1987); Reddy and Grieco, Int. J. Immunopharmacol., 9:483-488 (1987); EP Pub. 229,016 published July 15, 1987 (Biogen); and EP Pub. 171,128 published February 12, 1986 (Quidel)). In addition, Cavaille-Coll et al., Lancet, 1:1245 (1984) discusses exogenous IL-2 and mitogen responses in AIDS patients.

Many of the studies using IL-2 for treatment of AIDS were negative. See, e.g., Lotze et al., J. Immunol., 134:157-166 (1985) and Lane et al., J. Biol. Response Mod., 3:512-516 (1984); Volberding et al., AIDS Res. Hum. Retroviruses, 3:115-124 (1987).

In addition, the causes of the two different immunodeficiency diseases are different (human immunodeficiency virus infection versus a congenital immunodeficiency).

Accordingly, it is an object of the present invention to treat opportunistic infections caused by primary immunodeficiencies of the cellular immune function that are not a result of human immunodeficiency virus infection and that cannot be cured by bone marrow transplantations.

Summary of The Invention

This invention is directed to a method for treating infections in human pediatric patients that are a consequence of a primary immunodeficiency of cellular immune function in the patient and not a result of human immunodeficiency virus infection which cellular immune function can be shown to be improved in vitro through the addition of IL-2 to blood cells taken from the patient, which method comprises administering to the patient an immunotherapeutically effective amount of IL-2, wherein the IL-2 has substantially the amino acid sequence of native, mammalian IL-2.

In preferred aspects of the invention, the primary immunodeficiency is severe combined immunodeficiency syndrome or common variable immunodeficiency, and the infection is a viral, fungal, or bacterial infection.

In other preferred aspects, the IL-2 has substantially the amino acid sequence of mature native human IL-2, is recombinantly produced from microorganisms, and, further, is a mutein with one or more amino acids of the

mature, native human IL-2 sequence changed or deleted to improve the performance or purity of the IL-2.

Description of the Preferred Embodiments

Definitions:

As used herein, the term "immunotherapeutic" treatment refers to administration of IL-2 to the infected human pediatric patient. The treatment is not considered immunotherapeutic if after treatment the existing infection being treated is not eliminated or decreased.

As used herein, the term "immunotherapeutically effective amount" refers to the amount of IL-2 that is effective for immunotherapeutic treatment of the human patient. The exact optimum amount will depend on many factors, including the patient's clinical history and current infection, the schedule, the route, and the response of the patient. It has been found with the one patient tested that the IL-2 therapy must be continued to avoid reappearance of clinical signs of the infection. When the IL-2 treatment is discontinued, the signs of infection reappear; when the IL-2 treatment is resumed, symptoms of infection disappear.

As used herein, the term "infection" refers to any kind of infectious disease, including those caused by bacteria, fungi, viruses, protozoa, or parasites, provided that the infection is a consequence of a primary immunodeficiency of cellular immune function and not the result of human immunodeficiency virus infection. Examples of bacterial infections include P. aeruginosa, E. coli, tetanus, Mycobacterium species, Streptococcal strains, diphtheria, and Salmonella. Examples of fungal infections include cryptococcosis, histoplasmosis, and other infections due to Candida species. Examples of viral infections include Hepatitis A, measles virus, recurrent Herpes Simplex, the Rubella virus, the mumps virus, HIV-I or -II, Herpes Zoster, HTLV-I, HTLV-II, influenza, cytomegalovirus, chicken pox, and rhinoviruses. Preferably, the infection is bacterial, fungal or viral, more preferably viral. Most preferably, the infection is caused by chicken pox disease or cytomegalovirus.

As used herein, the term "primary immunodeficiency of cellular immune function" refers to a congenital or acquired (non-AIDS related) disorder in children that is characterized by defects of the pre- or intra-thymic cell or B cell development

adversely affecting both cellular and humoral defense to infectious agents. The defect is in mitogen-induced lymphocyte proliferation that is partially improved or augmented when exogenous IL-2 is added to the lymphocytes in vitro. This augmentation can be measured by whether the lymphocyte blastogenic responses to antigens and to phytohemagglutinin increase (improved cellular immune function in blood cells taken from the patient) or by clinical improvement, for example, whether the circulating antibody levels in the patient increase, or symptoms improve, or by both methods.

The types of primary immunodeficiencies that are within the scope of this invention include those classified according to the WHO classification system described by WHO (1979), Clin. Immunol. Immunopathol., 13:296, the disclosure of which is incorporated herein by reference. Such categories include, for example, various types of severe combined immunodeficiency syndromes (SCIDS), various types of Wiskott-Aldrich Syndromes (WAS), various types of X-linked agammaglobulinemia (X-linked a.), various types of immunodeficiencies with hyper-IgM (Hyper-IgM), various types of common variable immunodeficiencies (CVID), and the like. More preferred herein are SCIDS and CVID, and most preferred is SCIDS.

As used herein, the term "recombinant" refers to IL-2 produced by recombinant DNA techniques wherein generally the gene coding for the IL-2 is cloned by known recombinant DNA technology. For example, the human IL-2 gene is inserted into a suitable DNA vector such as a bacterial plasmid, preferably an E. coli plasmid, to obtain a recombinant plasmid, and the plasmid is used to transform a suitable host. The gene is expressed in the host to produce the recombinant protein. Examples of suitable recombinant plasmids for this purpose include pBR322, pCR1, pMB9 and pSC1. The transformed host may be eucaryotic or procaryotic, including mammalian, yeast, Aspergillus, and insect cells. One preferred embodiment herein, but not the only preferred embodiment, employs bacterial cells as the host.

As used herein, the term "pediatric" refers to children generally under the age of eighteen years. For treatment of CVID, the pediatric patient is typically initially treated in his or her teens, up to 15 years old. For treatment of SCIDS, the

patient is typically initially treated when he/she is 6 months to 2 years old and is treated throughout at least his/her adolescent years.

As used herein, the term "pharmaceutically acceptable" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient(s), is chemically inert, and is not toxic to the human patients to whom it is administered.

Modes of Carrying Out the Invention

The method of this invention involves administering to a human pediatric patient an immunotherapeutically effective amount of IL-2.

The administration may take place by any suitable technique, including parenteral administration. Examples of parenteral administration include intravenous, intraarterial, intramuscular, subcutaneous, and intraperitoneal, with intravenous, intramuscular, and subcutaneous administration being preferred, and intravenous being most preferred.

The dose and dosage regimen will depend mainly on the type of infection, the IL-2 (whether modified or not), the patient, the patient's history, and the patient's response to treatment. The amount must be effective to result in clinical improvement or in vitro evidence of immune function augmentation or both. The doses may be single doses or multiple doses. If multiple doses are employed, as preferred, the frequency of administration (schedule) will depend, for example, on the patient, type of infection, type of IL-2, dosage amounts, etc. For some types of infections, administration once a week may be effective, whereas for others, daily administration or administration every other day or every third day may be effective, but weekly administration ineffective. The practitioner will be able to ascertain upon routine experimentation which route of administration and frequency of administration are most effective in any particular case so as, in every case, to augment cellular immune function in vitro and/or improve clinical signs.

The dosage amount that appears to be most effective herein is one that results in no appearance and reappearance of infection and is not toxic or is acceptably toxic to the patient, as defined by the protocol in Example 1 below. Generally, such conditions as fever, chills, and general malaise are considered

acceptable. This optimum dose level will depend on many factors, for example, on the type of patient, the response of the patient, the type of infection, route and schedule of administration, existing infection burden, the type of IL-2, and the definition of toxicity. Toxicity to the human patient may be defined by the extent and type of side effects, with fever, chills, and general malaise considered acceptable toxicity for purposes herein.

If there is acceptable toxicity and if the route and schedule of administration are intravenous on a daily basis, the dosage level for each administration of underivatized recombinant, microbially produced IL-2 is preferably at least about 20,000 BRMP units/kg of patient weight, more preferably, about 20,000 to about 120,000 BRMP units/kg of patient weight, and most preferably about 30,000 to 70,000 BRMP units/kg of patient weight.

For parenteral administration the IL-2 will generally be formulated in a unit dosage injectable form (solution, suspension, or emulsion), preferably in a pharmaceutically acceptable carrier medium that is inherently non-toxic and non-therapeutic. Examples of such vehicles include saline, Ringer's solution, dextrose solution, mannitol, and normal serum albumin. Non-aqueous vehicles such as fixed oils and ethyl oleate may also be used. The carrier medium may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The IL-2 will typically be formulated in such carriers at a concentration of about 0.1 mg/mL to 100 mg/mL, preferably about 0.2 to 1 mg/mL.

Alternatively, the IL-2 may be made into a sterile, stable, lyophilized formulation in which the purified IL-2 is admixed with a water-soluble carrier such as mannitol, which provides bulk, and a sufficient amount of sodium dodecyl sulfate to ensure the solubility of the recombinant IL-2 in water. The formulation is suitable for reconstitution in aqueous injections for parenteral administration and it is stable and well-tolerated in human patients. The formulation method is more completely described in U.S. Pat. No. 4,604,377 issued August 5, 1986, the disclosure of which is incorporated herein by reference.

As mentioned above, the IL-2 herein may be any IL-2 prepared from tissue cultures or by recombinant techniques, and has substantially the amino acid sequence

of any mammalian IL-2, such as, e.g., mouse, rat, rabbit, primate, pig, and human. Preferably the IL-2 has substantially the native, human IL-2 amino acid sequence. More preferably, the IL-2 is recombinant IL-2 with substantially the native, human IL-2 amino acid sequence.

The recombinant IL-2 may be obtained as described by Taniguchi et al., Nature, 302:305-310 (1983) and Devos, Nucleic Acids Research, 11:4307-4323 (1983) by cloning the native human IL-2 gene and expressing it in transformed microorganisms. It may also be an IL-2 mutein as described in U.S. Patent No. 4,518,584, in which the cysteine normally occurring at position 125 of the wild-type or native molecule has been replaced by a neutral amino acid such as serine or alanine, or an IL-2 mutein as described in U.S. Patent No. 4,752,585, the disclosure of which is incorporated herein by reference, in which the methionine normally occurring at position 104 of the wild-type or native molecule has been replaced by a neutral amino acid such as alanine.

Preferably, the IL-2 is an unglycosylated protein that is produced by a microorganism that has been transformed with the human IL-2 cDNA sequence or a modified human cDNA sequence of IL-2 that encodes a protein with an amino acid sequence at least substantially identical to the amino acid sequence of native human IL-2, including the disulfide bond of the cysteines at positions 58 and 105, and has biological activity that is common to native human IL-2. Substantial identity of amino acid sequences means the sequences are identical or differ by one or more amino acid alterations (deletions, additions, or substitutions) that do not cause an adverse functional dissimilarity between the synthetic protein and native human IL-2. Examples of IL-2 proteins with such properties include those described by Taniguchi et al., Nature (1983), 302:305-310; by Devos, Nucleic Acids Research (1983), 11:4307-4323; and by European Patent Publication Nos. 91,539 and 88,195; in U.S. Patent 4,518,584, supra, EPA 86300333.1, and U.S. Patent No. 4,752,585, supra.

The hydrophobic recombinant IL-2 produced from certain transformed host cells containing recombinant DNA generally aggregates and/or precipitates inside the cell as opposed to being soluble in the cell culture medium. The intracellularly produced protein must be separated from the cellular debris and recovered from the cell before it can be formulated into a purified biologically active material. European

Pat. Publication No. 206,828 published December 30, 1986, the entire disclosure of which is incorporated herein by reference, discloses a process for isolating such a refractile material. In this process the cell membrane of the transformed host microorganism is disrupted, greater than 99% by weight of the salts is removed from the disruptate, the desalted disruptate is redisrupted, a material, preferably a sugar such as sucrose, is added to the disruptate to create a density or viscosity gradient in the liquid within the disruptate, and the refractile material is separated from the cellular debris by high-speed centrifugation, i.e., at about 10,000 to 40,000 x g. Preferably, the salts are removed from the disruptate by diafiltration or centrifugation and sucrose is added to increase the density of the liquid to about 1.1 to 1.3 g/mL.

After the centrifugation step, the pellet containing the refractile bodies is solubilized with a denaturant such as sodium dodecyl sulfate, the resulting suspension is centrifuged, and the supernatant containing the protein is processed to isolate the protein. The protein is separated from the supernatant by appropriate means such as reverse-phase high pressure liquid chromatography (RP-HPLC) and/or gel filtration chromatography. After such separation, the protein can be processed by disulfide exchange, e.g., using glutathione as described by the following references, the disclosures of all of which are incorporated herein by reference: Meth. Enzym. Vol. 131, Enzyme Structure Part L, C.H.W. Hirs, ed. (Academic Press, Inc., New York, 1986) P.83 (Creighton), Snyder, Biochemistry (1987) 26:688-694, and Saxena and Wetlaufer, Biochemistry (1970) 9:5015.

Alternatively, the separated protein may be oxidized (made to form disulfide bonds) to ensure the production of high yields of recombinant protein in a configuration most like its native counterpart. Such oxidation is described in U.S. Patent No. 4,530,787 to Z. Shaked et al., the disclosure of which is incorporated herein by reference. The oxidation may also be carried out by reacting an aqueous solution containing a solubilized form of the protein at a pH between about 5.5 and 9 in the presence of air with at least an effective amount of an oxidation promoter containing a Cu^{+2} cation, as described in U.S. Patent No. 4,572,798 to K. Koths et al., the disclosure of which is incorporated herein by reference. The preferred oxidation promoter or oxidant is $CuCl_2$ or (o-phenanthroline)$_2$ Cu^{+2}. After oxidation, the protein may optionally be desalted and purified further by RP-HPLC, dilution/diafiltration,

S200 gel filtration chromatography, and ultrafiltration techniques before modification with activated homopolymer as described further hereinbelow.

The polymer modification may be carried out at any step after the heterologous IL-2 protein has been isolated in sufficiently pure form to be biologically active for therapeutic purposes. The point at which the modification will occur will depend, for example, on the ultimate purity of the IL-2 required for the final use thereof, including pharmaceutical formulation.

Guanidine hydrochloride may be used as a denaturant for the solubilization of the particle paste or after the HPLC step, as described more fully in PCT/US88/01043 and PCT/US88/01064, both of which were filed on March 31, 1987, and the disclosures of both of which are incorporated herein by reference.

Briefly, PCT/US88/01043 describes and claims a process for recovering purified recombinant IL-2 from a transformed microorganism comprising:

(a) disrupting the cell membrane of the microorganism;

(b) separating water-insoluble IL-2-containing material from the disruptate;

(c) mixing the insoluble IL-2-containing material of step (b) at a pH of about 7 to about 9 with an aqueous solution of a reducing agent and a chaotropic agent whereby the IL-2 in the insoluble material is dissolved and denatured;

(d) separating the IL-2-containing solution of step (c) from the undissolved portion of the insoluble material;

(e) removing the reducing agent from the separated IL-2-containing solution;

(f) oxidizing the IL-2 in the solution while maintaining the concentration of chaotropic agent at a strongly denaturing concentration, whereby the natural disulfide bridge of IL-2 is formed;

(g) after the oxidation of step (f) is complete, diluting the solution to reduce the concentration of chaotropic agent in the solution to a level at which the oxidized IL-2 is permitted to renature and a precipitate forms;

(h) separating the precipitate from the solution to provide a supernatant:

(i) purifying the oxidized IL-2 in the supernatant by (1) reverse-phase high performance liquid chromatography followed by dissolution of the precipitate resulting from the chromatography in a solution of chaotropic agent and removal of the chaotropic agent from the solution, or (2) hydrophobic interaction chromatography followed by ion exchange chromatography; and

(j) recovering a purified oxidized heterologous human IL-2 composition having an IL-2 content of at least about 95% as determined by reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis, a solubility in phosphate buffered saline of at least about 5 mg IL-2 per mL, a specific activity of at least about 1 x 10^6 units/mg as determined by HT-2 cell proliferation assay, and an endotoxin content of less than about 0.1 nanograms per mg of IL-2.

The chaotropic agent is preferably guanidine hydrochloride and the strongly denaturing concentration is preferably at least about 6M.

Briefly, PCT/US88/01064 describes and claims a process for recovering recombinant IL-2 from transformed microorganisms containing the IL-2 wherein the IL-2 is separated from the bulk of the cellular components of the microorganisms, solubilized in a reduced form, thereafter oxidized, and thereafter purified to clinically acceptable purity and endotoxin levels, the improvement in which process comprises denaturing the oxidized, purified IL-2 by placing the IL-2 in a solution of a chaotropic agent, removing solids from the solution, and thereafter renaturing the IL-2 from the solution, whereby a renatured, oxidized, purified IL-2 having improved stability and solubility properties in the absence of detergents is obtained. Preferably the solubilization of the reduced IL-2 is achieved by mixing the separated IL-2 with an aqueous solution from 0.1 to 10% (w/v) of sodium dodecyl sulfate. More preferably, the solution of a chaotropic agent is a 4 to 8 M aqueous guanidine hydrochloride solution.

Preferably, the mutein herein is selected from ser_{125}IL-2, $ala_{104}ser_{125}$IL-2, des-ala_1IL-2, des-ala_1ala_{104}IL-2, des-$ala_1ala_{104}ser_{125}$IL-2, des-ala_1ser_{125}IL-2, or ala_{104}IL-2. Most preferably, the IL-2 is the des-ala_1ser_{125}IL-2 mutein in which the initial terminal alanine is deleted and the cysteine at position 125 is replaced by a serine residue. The IL-2 employed may have at least one or more of the first five N-terminal amino acids of the native IL-2 deleted, such as the muteins disclosed in U.S. Patent No.

4,752,585. More preferred is the $ala_{104}ser_{125}$ IL-2 mutein which has the first four N-terminal amino acids deleted.

The IL-2 may be purified to clinical purity by the method described in U.S. Patent No. 4,569,790, issued February 11, 1986, the disclosure of which is incorporated herein by reference.

In an alternative formulation, the recombinant, E.-coli-produced IL-2 may be chemically modified to increase its solubility and circulating half-life, so that it may be administered less often at lower doses to the patient, For example, U.S. Patent No. 4,766,106, the disclosure of which is incorporated herein by reference, describes solubilizing the IL-2, not by a detergent, but by reacting the IL-2 with an activated polymer selected from polyethylene glycol homopolymers and polyoxyethylated polyols. The polymer is activated by conjugation with a coupling agent having terminal groups reactive with both the free amino or thiol groups of the IL-2 and the hydroxyl group of the polymer. Examples of such coupling agents include hydroxynitrobenzene sulfonic ester, cyanuric acid chloride, and N-hydroxysuccinimide. This modification eliminates the necessity for adding detergents to solubilize the IL-2 at physiological pH. The IL-2 is then formulated directly with the water-soluble carrier and buffer as described above, and the formulation may be lyophilized and the lyophilized mixture reconstituted as described above.

In still another alternative formulation, the recombinant, E.-coli-produced IL-2 is covalently conjugated to a polyproline molecule through a flexible spacer arm as described in PCT/US87/02930 filed November 10, 1987, the disclosure of which is incorporated herein by reference. In still another alternative, the recombinant, E. coli-produced IL-2 may be conjugated via at least one of its lysine residues to a heparin fragment having a terminal 2,5-anhydro-D-mannose residue through its aldehyde group, as described by U.S. Patent 4,745,180, the disclosure of which is incorporated herein by reference.

When the IL-2 is so modified, administration is expected to be at a dose of between only about 1000 and 60,000 BRMP units/kg of patient weight at least only twice a month, as opposed to at least once a week, due to the increased circulatory half-life of such modified IL-2 over unmodified IL-2.

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner. In these examples all parts for solids are by weight and all percentages for liquids and gases are by volume, unless otherwise noted, and all temperatures are given in degrees Celsius.

EXAMPLE 1

General Treatment Plan

1. Patient

One patient, age 17 months, was admitted for a Phase I study. She met the following criteria for entering the study:

1. She had documented primary immunodeficiency syndrome (SCIDS) with recurrent and severe life-threatening viral infections, including chicken pox, despite high dose gammaglobulin and Acyclovir (9-(2-hydroxyethoxy methyl) guanine). Her in vitro proliferative response to mitogen, total lymphocyte count, T4 and T8 lymphocyte subsets, as well as in vitro IL-2 and antibody production, were very low.
2. Her guardian gave written, informed consent.
3. She had a minimum life expectancy of 12 weeks.
4. She had failed prior attempts to restore immunocompetence by bone marrow transplantation.

Patients excluded from the study are:

1. Those who have an HLA-MLC compatible donor.
2. Those considered ineligible by their pediatrician.
3. Those participating in another clinical trial aimed at the treatment of their immune deficiency within three weeks of entry onto this study.
4. Those eligible for a protocol of higher priority for this disease.
5. Those who are not considered fully recovered from any prior surgical procedures.
6. Those who are HIV antibody or antigen positive.

2. IL-2

The recombinant IL-2 employed in this example was des-ala$_1$ser$_{125}$IL-2. The amino acid sequence of this IL-2 differs from the amino acid sequence of native human IL-2 in that it lacks the initial alanine of the native molecule, and the cysteine at position 125 has been changed to serine. Samples of E. coli that produce this IL-2 have been deposited by Cetus Corporation in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA on September 26, 1983 under accession number 39,452 and on March 6, 1984 under accession number 39,626 under the provisions of the Budapest Treaty.

The IL-2 was processed and purified as described in the text and Figure 1 of U.S. Patent No. 4,604,377, the disclosure of which is incorporated herein by reference, except that the oxidation was carried out using copper chloride, as described in U.S. Patent No. 4,572,798 rather than o-iodosobenzoate. When the IL-2 was recovered from the chromatography step(s), it was lyophilized and resuspended in a neutral aqueous buffer containing the reducing agent (dithiothreitol) to keep the IL-2 in a reduced state and a solubilizing agent to keep it in solution. The purity of the recombinant IL-2 after the chromatography step(s) was at least about 95%, and the IL-2 contained less than about 0.02 ng/mL endotoxin as determined by the Limulus amebocyte assay.

The purified IL-2 was formulated at a concentration of 0.3 mg/mL with 50 mg/mL mannitol.

3. Treatment Plan

The patient received a daily intravenous infusion of the IL-2 diluted in 15-20 mL of 5% dextrose in water administered over 60 minutes. The patient was treated for 6-8 weeks under the following dose escalation schema:

Week	Dose of IL-2 (BRMP units/kg patient weight)	(Cetus units/kg patient weight)
1 & 2	23,000	10,000
3	46,000	20,000
4	69,000	30,000
5	69,000	40,000
6	69,000	50,000
7 & on	69,000	60,000

Dose escalation would be stopped if toxicity was observed or the patient's proliferative response to mitogen became normal.

4. Results

After the 2nd week at 23,000 units, the patient exhibited no response in immune reconstitution. After the third week of treatment at 46,000 units, she showed some improvement in proliferative responses to phytomitogen (PH-A) and Con A antigen, and total numbers of T and B cells.

This patient exhibited, at 30,000 units, a normal PH-A and Con A response and a normal T and B cell number. She also achieved an antibody production in vitro.

When the patient was taken off IL-2 for two weeks, her in vitro studies showed a PH-A response of 35% of normal, and a normal Con A and normal pokeweed mitogen response. Her in vitro production of antibodies became abnormal when she was taken off IL-2.

When the patient was treated again with IL-2 at 69,000 units, her in vitro responses became normal and in vitro antibody production levels were normal.

During her hospitalization the patient had a few febrile episodes that were resolved with antibiotic treatment. In regard to her feedings, she underwent gastrostomy placement and a Broviac catheter placement X2 and was placed on total parenteral nutrition (TPN) as well as gastrostomy feedings that she tolerated well. She was placed on Pregestimil brand iron-fortified protein hydrolysate formula (Mead

Johnson Nutritional Division of Bristol-Myers), at 30 cc per hour without diarrhea or vomiting. She also received supplemental TPN. She had a documented moderate gastroesophageal reflux and esophagitis that responded to Reglan brand metochlopramide hydrochloride (A.H. Robins Co.) and cimetadine, a histamine H_2 receptor antagonist (obtainable under the name Tagamet® from SmithKline Beckman Corporation).

While still hospitalized the patient was found to have normal skin turgor with few maculopopular rashes around her neck and upper shoulder regions. The chest was clear without rales or rhonchi. The abdomen was full with palpable liver about 2 cm down. On the diaper area there was a hemorrhagic rash that improved over time with the use of a hydrocortisone product and an antibiotic cream. The patient had a urine that was positive for cytomegalovirus.

The patient was allowed to go home and receive daily intravenous bolus injections of IL-2 over a one-hour period. None of the clinical signs of infection returned. When the schedule was reduced to twice a week, the clinical signs started to reappear.

In summary, the treatment of the child with IL-2 resulted in no evidence of severe toxicity clearly attributable to administration of IL-2. Increases in systolic blood pressure and appearance of tachycardia had occurred prior to any infusions of IL-2. Also, the deficiency of the proliferative response to PHA stimulation of the patient's lymphocytes and the abnormality of her lymphocytes to be unable to make Ig responsively to stimulation with pokeweed mitogen also appeared to be corrected by IL-2 treatment.

Further, upon IL-2 treatment, the number of B cells decreased from high to normal levels and the numbers of total T lymphocytes and T helper and T suppressor cells increased from low numbers to numbers within the normal range. These increased responses did not correct the patient's inability to produce IL-2.

After continuous IL-2 treatment for approximately 13 months, the patient had normal in vitro responses for PH-A, Con A, and pokeweed mitogen. Her in vivo responses included: an absence of infections in a non-sterile environment (i.e., living at home); growth restoration; and no hospitalization.

EXAMPLE 2

A second patient, who fulfilled many of the criteria shown in Example 1, had a variant of severe combined immunodeficiency disease. His history showed that he developed pneumonia at 4 months, so that he was hospitalized and treated with antibiotics. The patient had low blood counts and at 9 months was hypoplastic. At 10 months, he became aplastic and was treated with steroids, which induced full hematological recovery. Repeated infections led to an immunological examination between 10 months and 18 months, which revealed depressed T and B cell functions. At the age of 1-1/2 years, another immunological examination revealed low immunoglobulin levels and no T cell functions in vitro. He continued to have infections and failure to thrive prior to IL-2 treatment. The family was tested for HLA typing, but no members were found to be histocompatible; so a transplant was not recommended. His presenting symptoms were: sinusitis; thrush; pneumonia; sepsis; hypogammaglobulinemia; hepatomegaly; aplastic anemia; and failure to thrive. IL-2 treatment began on December 11, 1987 according to the following dose schedule and escalated to 40,000 units/kg.

Week	Dose of IL-2 Dose per day (Cetus units/kg patient weight)
1	10,000
2	10,000
3	20,000
4	30,000
5	40,000
6	50,000

His in vitro mitogen responses were improved at 4 weeks of therapy which showed response to the treatment. The in vitro parameters continued to improve on treatment and clinically, all his other symptoms were resolved, such as a steady weight gain, control of thrush and no serious infections while on therapy. He was in a sterile environment during this therapy, so he was not challenged with a non-sterile environment. The patient continued on treatment until March 17, 1988 and was

removed from the study at the request of his mother despite immunologic improvement. Since the cessation of IL-2, the patient has been hospitalized frequently for infections and he has declined nutritionally.

EXAMPLE 3

A third patient who fulfilled many of the Example 1 criteria was a 20 month old boy when he began IL-2 treatment. He was diagnosed with severe combined immunodeficiency disease and his presenting symptoms were fungal infections, pneumocystis, anemia, sepsis, diarrhea, and a groin rash. He had a prior haploidentical bone marrow transplant which failed. Prior to IL-2 treatment, he had 100% host cells. He was treated under the dose schedule noted in Example 2. His clinical responses showed the resolution of a fungal nail bed infection, and the improvement or resolution of his presenting symptoms. His in vitro responses showed an increase in soluble T cell receptor levels, and increases in T cell and NK cell numbers (all host).

EXAMPLE 4

A fourth patient who fulfilled many of the Example 1 criteria was a 10 month old boy diagnosed with a combined immunodeficiency syndrome characterized by failure to produce interleukin 2 (IL-2), impaired proliferative responses to agents which activate T cells via the T cell receptor (i.e., T cell mitogens, OKT3 and antigens) and to a protein kinase C activator (i.e., phorbal myristate acetate) when used alone, as well as by a severe B cell defect. He had a succession of serious infectious illnesses, beginning with Group B streptococcal septicemia shortly after birth, followed by life-threatening infections with Klebsiella (septicemia), staphylococcus (impetigo), respiratory syncytial virus (pneumonia), and pericarditis.

These led to pericardectomy, enterococci (sepsis), P. carinii, cytomegalovirus (hepatitis), rotavirus and papovavirus (necrotizing skin lesions). He began intravenous IL-2 therapy according to the schedule in Example 2 and the dosage was gradually increased to 40,000 units/kg/24 hours after 5 weeks. IL-2 therapy was well tolerated at all times, and he showed no adverse effects from the IL-2 therapy. His immune function and clinical status improved greatly, such that

after approximately 2-1/2 months, he had normal lymphocyte proliferative responses to T cell mitogens, all skin lesions were nearly healed, he was afebrile, had no diarrhea and was eating well. After 3 months, his IL-2 therapy was spaced to every other day in preparation for anticipated discharge and subsequent home IL-2 infusions.

The next day he developed a low grade fever. Blood cultures were obtained and antibodies started. The cultures were negative. Several days later, right parotid swelling and rhinorrhea were found and an ENT consult was done. The findings from this consultation indicated no respiratory distress with probable parotitis even though no purulent material was expressed. The patient's clinical course deteriorated; so he was transferred to the acute care unit where he experienced a series of cardiac arrests and expired. The probable cause of death was determined to be pseudomonas sepsis. Neither death, nor the events which led to it were judged to be due to an adverse effect of IL-2 therapy.

Modifications of the above-described modes for carrying out the invention that are obvious to those skilled in the fields of molecular and clinical biology, pharmacology, and related fields are intended to be within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for treating infections in human pediatric patients that are a consequence of a primary immunodeficiency of cellular immune function in the patient, and not a result of human immunodeficiency virus infection, which cellular immune function can be shown to be improved in vitro through the addition of interleukin-2 (IL-2) to blood cells taken from the patient, which method comprises administering to the patient an immunotherapeutically effective amount of human IL-2, or muteins thereof.

2. The method of Claim 1 wherein the primary immunodeficiency is severe combined immunodeficiency syndrome.

3. The method of Claim 1 wherein the primary immunodeficiency is common variable immunodeficiency.

4. The method of Claim 1 wherein the patient is a child up to fifteen years old.

5. The method of Claim 1 wherein the IL-2 is administered parenterally.

6. The method of Claim 5 wherein the IL-2 is administered at least once a week intravenously.

7. The method of Claim 6 wherein the IL-2 is administered in an amount of at least about 20,000 BRMP units per kg of patient weight.

8. The method of Claim 7 wherein the IL-2 is administered at doses between about 20,000 and about 120,000 BRMP units per kg of patient weight on a daily schedule, which doses can be shown to result in clinical improvement or in improvement of cellular immune function in vitro or both.

9. The method of Claim 1 wherein the IL-2 has substantially the amino acid sequence of native, human IL-2 and is produced by recombinant DNA technology.

10. The method of Claim 9 wherein the IL-2 is a mutein.

11. The method of Claim 10 wherein the mutein has a serine or alanine residue at position 125, numbered in accordance with the mature, human native sequence.

12. The method of Claim 11 wherein the mutein has no alanine residue at position 1, numbered in accordance with the mature, human native sequence.

13. The method of Claim 10 wherein the mutein has a serine or alanine residue at position 104, numbered in accordance with the mature, human native sequence.

14. The method of Claim 10 wherein the mutein is des-ala_1IL-2, des-ala_1ala_{104}IL-2, des-$ala_1ala_{104}ser_{125}$IL-2, des-ala_1ser_{125}IL-2, ala_{104}IL-2, ser_{125}IL-2, or $ala_{104}ser_{125}$IL-2.

15. The method of Claim 14 wherein the mutein is des-ala_1ser_{125}IL-2.

16. The method of Claim 1 wherein the IL-2 is chemically modified to increase its solubility and circulating half-life.

17. The method of Claim 16 wherein the IL-2 is covalently conjugated to a homopolymer of polyethylene glycol, polyproline, a heparin fragment, or a polyoxyethylated polyol, wherein the polyethylene glycol homopolymer may be unsubstituted or substituted with an alkyl group on one end.

18. The method of Claim 16 wherein the chemically modified IL-2 is administered at least twice per month at doses between about 1000 and 60,000 BRMP units per kg of patient weight.